

Sequence Listing

<110> Ajinomoto Co. Inc.

5 <120> Method of constructing amino acid producing bacteria, and method of preparing amino acids by fermentation with the constructed amino acid producing bacteria

<130> OP 99052

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<151> 1998-9-25

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20 ttc gcg atg atc cgc gat ggc gtg gca tct tat ttg aac gac tca gat 96

Phe Ala Met Ile Arg Asp Gly Val Ala Ser Tyr Leu Asn Asp Ser Asp

20

25

30

ccg gag gag acc aac gag tgg atg gat tca ctc gac gga tta ctc cag 144

25 Pro Glu Glu Thr Asn Glu Trp Met Asp Ser Leu Asp Gly Leu Leu Gln

35

40

45

gag tct tct cca gaa cgt gct cgt tac ctc atg ctt cgt ttg ctt gag 192

Glu Ser Ser Pro Glu Arg Ala Arg Tyr Leu Met Leu Arg Leu Leu Glu

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cgt gca tct gca aag cgc gta tct ctt ccc cca atg acg tca acc gac 240

Arg Ala Ser Ala Lys Arg Val Ser Leu Pro Pro Met Thr Ser Thr Asp

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75

80

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Tyr Val Asn Thr Ile Pro Thr Ser Met Glu Pro Glu Phe Pro Gly Asp
 85 90 95
 gag gaa atg gag aag cgt tac cgt cgt tgg att cgc tgg aac gca gcc 336
 5 Glu Glu Met Glu Lys Arg Tyr Arg Arg Trp Ile Arg Trp Asn Ala Ala
 100 105 110
 atc atg gtt cac cgc gct cag cga cca ggc atc ggc gtc ggc gga cac 384
 Ile Met Val His Arg Ala Gln Arg Pro Gly Ile Gly Val Gly Gly His
 10 115 120 125
 att tcc act tac gca ggc gca gcc cct ctg tac gaa gtt ggc ttc aac 432
 Ile Ser Thr Tyr Ala Gly Ala Ala Pro Leu Tyr Glu Val Gly Phe Asn
 130 135 140
 15 cac ttc ttc cgc ggc aag gat cac cca ggc ggc ggc gac cag atc ttc 480
 His Phe Phe Arg Gly Lys Asp His Pro Gly Gly Gly Asp Gln Ile Phe
 145 150 155 160
 20 ttc cag ggc cac gca tca cca ggt atg tac gca cgt gca ttc atg gag 528
 Phe Gln Gly His Ala Ser Pro Gly Met Tyr Ala Arg Ala Phe Met Glu
 165 170 175
 ggt cgc ctt tct gaa gac gat ctc gat ggc ttc cgt cag gaa gtt tcc 576
 25 Gly Arg Leu Ser Glu Asp Asp Leu Asp Gly Phe Arg Gln Glu Val Ser
 180 185 190
 cgt gag cag ggt ggc att ccg tcc tac cct cac cca cac ggt atg aag 624
 Arg Glu Gln Gly Gly Ile Pro Ser Tyr Pro His Pro His Gly Met Lys
 30 195 200 205
 gac ttc tgg gag ttc cca act gtg tcc atg ggt ctt ggc cca atg gat 672
 Asp Phe Trp Glu Phe Pro Thr Val Ser Met Gly Leu Gly Pro Met Asp
 210 215 220
 35 gcc att tac cag gca cgt ttc aac cgc tac ctc gaa aac cgt ggc atc 720

Ala Ile Tyr Gln Ala Arg Phe Asn Arg Tyr Leu Glu Asn Arg Gly Ile
225 230 235 240

aag gac acc tct gac cag cac gtc tgg gcc ttc ctt ggc gac ggc gaa 768
5 Lys Asp Thr Ser Asp Gln His Val Trp Ala Phe Leu Gly Asp Gly Glu
245 250 255

atg gac gag cca gaa tca cgt ggt ctc atc cag cag gct gca ctg aac 816
Met Asp Glu Pro Glu Ser Arg Gly Leu Ile Gln Gln Ala Ala Leu Asn
10 260 265 270

aac ctg gac aac ctg acc ttc gtg gtt aac tgc aac ctg cag cgt ctc 864
Asn Leu Asp Asn Leu Thr Phe Val Val Asn Cys Asn Leu Gln Arg Leu
275 280 285

gac gga cct gtc cgc ggt aac acc aag atc atc cag gaa ctc gag tcc 912
15 Asp Gly Pro Val Arg Gly Asn Thr Lys Ile Ile Gln Glu Leu Glu Ser
290 295 300

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20 Phe Phe Arg Gly Ala Gly Trp Ser Val Ile Lys Val Val Trp Gly Arg
305 310 315 320

gag tgg gat gaa ctt ctg gag aag gac cag gat ggt gca ctt gtt gag 1008
Glu Trp Asp Glu Leu Leu Glu Lys Asp Gln Asp Gly Ala Leu Val Glu
25 325 330 335

atc atg aac aac acc tcc gat ggt gac tac cag acc ttc aag gct aac 1056
Ile Met Asn Asn Thr Ser Asp Gly Asp Tyr Gln Thr Phe Lys Ala Asn
340 345 350

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30 Asp Gly Ala Tyr Val Arg Glu His Phe Phe Gly Arg Asp Pro Arg Thr
355 360 365

gca aag ctc gtt gag aac atg acc gac gaa gaa atc tgg aag ctg cca 1152
35 Ala Lys Leu Val Glu Asn Met Thr Asp Glu Glu Ile Trp Lys Leu Pro

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5 385 390 395 400

405 410 415

420 425 430

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450 455 460

25 465 470 475 480

485 490 495

500 505 510

Lys Gly Ser Gly Lys Gln Gln Ile Ala Thr Thr Met Ala Thr Val Arg

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acc ttc aag gaa ctg atg cgc gat aag ggc ttg gct gat cgc ctt gtc 1632
 Thr Phe Lys Glu Leu Met Arg Asp Lys Gly Leu Ala Asp Arg Leu Val

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cca acc ttg aag atc tac aac ccg cac ggt cag aac tac gtg cct gtt 1728
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ctg cac gaa ggc atc aac gag gct ggt tcc gtg gca tcg ttc atc gct 1824
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640

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gca gca gcc gat cag atg gca cgt ggc ttc ctc ttg ggc gct acc gca 1968
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655

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 Phe Val Thr Thr Gln Leu Lys Gln Thr Ser Gly Pro Tyr Val Ala Val
 5 820 825 830
 tct gac ttc tcc act gat ctg cca aac cag atc cgt gaa tgg gtc cca 2544
 Ser Asp Phe Ser Thr Asp Leu Pro Asn Gln Ile Arg Glu Trp Val Pro
 835 840 845
 10 ggc gac tac acc gtt ctg ggt gca gat ggc ttc ggt ttc tct gat acc 2592
 Gly Asp Tyr Thr Val Leu Gly Ala Asp Gly Phe Gly Phe Ser Asp Thr
 850 855 860
 15 cgc cca gct gct cgt cgc ttc ttc aac atc gac gct gag tcc att gtt 2640
 Arg Pro Ala Ala Arg Arg Phe Phe Asn Ile Asp Ala Glu Ser Ile Val
 865 870 875 880
 20 gtt gca gtg ctg aac tcc ctg gca cgc gaa ggc aag atc gac gtc tcc 2688
 Val Ala Val Leu Asn Ser Leu Ala Arg Glu Gly Lys Ile Asp Val Ser
 885 890 895
 gtt gct gct cag gct gct gag aag ttc aag ttg gat gat cct acg agt 2736
 Val Ala Ala Gln Ala Ala Glu Lys Phe Lys Leu Asp Asp Pro Thr Ser
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<213> Brevibacterium lactofermentum ATCC13869

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560 565 570

5 aac tac gtg cct gtt gac cac gac ctg atg ctc tcc tac cgt gag gca 4120
 Asn Tyr Val Pro Val Asp His Asp Leu Met Leu Ser Tyr Arg Glu Ala

575 580 585

10 cct gaa gga cag atc ctg cac gaa ggc atc aac gag gct ggt tcc gtg 4168
 Pro Glu Gly Gln Ile Leu His Glu Gly Ile Asn Glu Ala Gly Ser Val

590 595 600

15 gca tcg ttc atc gct gcg ggt acc tcc tac gcc acc cac ggc aag gcc 4216
 Ala Ser Phe Ile Ala Ala Gly Thr Ser Tyr Ala Thr His Gly Lys Ala

605 610 615

20 atg att ccg ctg tac atc ttc tac tcg atg ttc gga ttc cag cgc acc 4264
 Met Ile Pro Leu Tyr Ile Phe Tyr Ser Met Phe Gly Phe Gln Arg Thr

620 625 630 635

ggt gac tcc atc tgg gca gca gcc gat cag atg gca cgt ggc ttc ctc 4312
 Gly Asp Ser Ile Trp Ala Ala Ala Asp Gln Met Ala Arg Gly Phe Leu

640 645 650

25 ttg ggc gct acc gca ggt cgc acc acc ctg acc ggt gaa ggc ctc cag 4360
 Leu Gly Ala Thr Ala Gly Arg Thr Thr Leu Thr Gly Glu Gly Leu Gln

655 660 665

30 cac atg gat gga cac tcc cct gtc ttg gct tcc acc aac gag ggt gtc 4408
 His Met Asp Gly His Ser Pro Val Leu Ala Ser Thr Asn Glu Gly Val

670 675 680

35 gag acc tac gac cca tcc ttt gcg tac gag atc gca cac ctg gtt cac 4456
 Glu Thr Tyr Asp Pro Ser Phe Ala Tyr Glu Ile Ala His Leu Val His

685 690 695

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5 tac tac atc acc atc tac aac gag cca acc cca cag cca gct gag cca 4552
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 720 725 730

10 gaa gga ctg gac gta gaa ggc ctg cac aag ggc atc tac ctc tac tcc 4600
 Glu Gly Leu Asp Val Glu Gly Leu His Lys Gly Ile Tyr Leu Tyr Ser
 735 740 745

15 cgc ggt gaa ggc acc ggc cat gag gca aac atc ttg gct tcc ggt gtt 4648
 Arg Gly Glu Gly Thr Gly His Glu Ala Asn Ile Leu Ala Ser Gly Val
 750 755 760

20 ggt atg cag tgg gct ctc aag gct gca tcc atc ctt gag gct gac tac 4696
 Gly Met Gln Trp Ala Leu Lys Ala Ala Ser Ile Leu Glu Ala Asp Tyr
 765 770 775

25 gga gtt cgt gcc aac att tac tcc gct act tct tgg gtt aac ttg gct 4744
 Gly Val Arg Ala Asn Ile Tyr Ser Ala Thr Ser Trp Val Asn Leu Ala
 780 785 790 795

30 cgc gat ggc gct gct cgt aac aag gca cag ctg cgc aac cca ggt gca 4792
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 800 805 810

35 gat gct ggc gag gca ttc gta acc acc cag ctg aag cag acc tcc ggc 4840
 Asp Ala Gly Glu Ala Phe Val Thr Thr Gln Leu Lys Gln Thr Ser Gly
 815 820 825

cca tac gtt gca gtg tct gac ttc tcc act gat ctg cca aac cag atc 4888
 Pro Tyr Val Ala Val Ser Asp Phe Ser Thr Asp Leu Pro Asn Gln Ile
 830 835 840

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ggt ttc tct gat acc cgc cca gct gct cgt cgc ttc ttc aac atc gac 4984
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905

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tagacaatct ggccttcatt catcatgatc aggcgattgc ccaggcgaat tgectgttcc 5370
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 amplification plasmid

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 amplification plasmid

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 gene LA promoter

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gene LA promoter

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30 <220> primer for introducing a mutation of *Brevibacterium lactofermentum* pdhA
gene LA promoter

<400> 39

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<211> 38

<212> nucleic acid

<220> primer for introducing a mutation of *Brevibacterium lactofermentum* pdhA
gene LA promoter

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gene LA promoter

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10 <210> 51

<211> 40

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